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Source: Journal of Economic Entomology, 97(6):1906-1911.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/0022-0493-97.6.1906
URL: http://www.bioone.org/doi/full/10.1603/0022-0493-97.6.1906

Effects of Two Biorational Insecticides, Spinosad and Methoxyfenozide, on *Spodoptera littoralis* (Lepidoptera: Noctuidae) Under Laboratory Conditions

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J. Econ. Entomol. 97(6): 1906–1911 (2004)

ABSTRACT The toxicity of two biorational insecticides, spinosad (Tracer) and methoxyfenozide (RH-2485), was tested against eggs, larvae, and pupae of the noctuid *Spodoptera littoralis* (Boisduval). In the first experiment, filter paper circles containing egg masses of two different age classes, young (<24 h old) and old (24–48 h old), were dipped in different concentrations of each insecticide diluted in either water or acetone. No ovicidal activity was recorded when insecticides were diluted in water. In contrast, when insecticides were diluted in acetone, both egg age classes generally showed a concentration-dependent response for both compounds. Mortality of larvae that hatched from both egg age classes was significantly increased, compared with control larvae, at all concentrations of both insecticides when diluted in water or acetone alike. The prevalence of mortality was similar with each insecticide. In the second experiment, third instars of *S. littoralis* were fed semisynthetic diet containing different concentrations of both insecticides. According to LC_{50} values, no significant differences were observed between spinosad (2.11 mg [AI]/kg diet) and methoxyfenozide (3.98 mg [AI]/kg diet) after 48 h of treatment, based on the overlap of 95% CL. Toxic effects on the mortality of pupae, adult emergence, and the prevalence of deformed adults after topical application on young pupae also were examined. Only methoxyfenozide caused pupal mortality and deformed adults. Our results suggest that spinosad and methoxyfenozide are potentially potent compounds for control of *S. littoralis*.

KEY WORDS *Spodoptera littoralis*, spinosad, methoxyfenozide, toxicity, mortality

THE NOCTUID *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered the most serious pest of Egyptian cotton (*Gossypium* spp.). Larvae of this pest also attack other crops such as vegetables, ornamentals, and orchard trees (Bayoumi et al. 1998). The geographical range of this species includes the Mediterranean region, most of the Middle East, and north and central Africa. In Spain, *S. littoralis* causes serious crop damage in southern regions (Carter 1984, Gómez and Arroyo 1994). After intensive use of broad-spectrum insecticides, *S. littoralis* populations have developed high levels of resistance to organophosphates, carbamates, and pyrethroids (Ishaaya et al. 1995). Moreover, biological insecticides such as *Bacillus thuringiensis* Berliner have been reported to provide inadequate control of *S. littoralis* (Smagghe et al. 1999).

To decrease the environmental impact of crop protection measures, there is a recognized need to find alternatives for the control of *S. littoralis* that are compatible with integrated pest management (IPM) practices (Adán et al. 1996). Biorational control agents, based on naturally derived products or compounds that disrupt the physiological processes of insects have attracted particular interest. Several new chemistries with unique modes of action have recently been developed and may soon become available for *S. littoralis* control. These compounds include spinosad and methoxyfenozide.

Spinosad (Dow AgroSciences LLC, Ibérica Co., Madrid, Spain) primarily comprises two insecticidal neurotoxic macrocyclic lactones, spinosyn A and D, that are secondary metabolites produced by the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao (Sparks et al. 1998, Moulton et al. 1999). Routes of entry include topical and ingestion, and this product is particularly effective against Lepidoptera, Diptera, and Thysanoptera (Bret et al. 1997). Due to its selective action, spinosad seems to be one of the most judicious insecticides available for the conservation of insect predator populations (Williams et al. 2003), although certain predator species and hymenopteran

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0022-0493/04/1906–1911$04.00/0

parasitoids seem to be susceptible to spinosad intoxication (Cisneros et al. 2002, Schneider et al. 2003a). The mode of action of this compound involves the postsynaptic nicotinic acethylcholine receptor and the GABA receptor (Salgado 1997, Watson 2001). Due to its high activity at low rates, spinosad is commonly used in the United States for the control of lepidopteran pests in cotton (Saunders and Bret 1997) and has recently been registered in Spain for use against pepper and tomato pests in greenhouses.

Methoxyfenozide belongs to new group of insect growth regulators, the molting accelerating compounds (MACs), that have potential for the control of Lepidoptera (Palli and Retnakaran 2001). This compound mimics the molting hormone by inducing a premature and lethal molt by direct stimulation of the ecdysteroid receptors (Dhadialla et al. 1998). In addition, no adverse effects of methoxyfenozide on mammals, birds, fishes, others vertebrates, and various beneficial insects have been reported (Aller and Ramsay 1988; Schneider and Viñuela 1999; Schneider et al. 2003a, 2003b).

The aim of the current study was to determinate the effects of spinosad and methoxyfenozide on the immature stages (eggs, larvae, and pupae) of *S. littoralis* to evaluate possible differences in the toxicity of these products and their potential as control agents in IPM programs.

Materials and Methods

Insect Rearing. *S. littoralis* used in these tests came from a colony maintained in our laboratory with no history of insecticide exposure. Larvae were reared on a semisynthetic diet (Poitout and Bues 1974) in a controlled environmental chamber at 25 ± 2°C, 75 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Adults were fed with a 15% solution of honey. Filter paper was provided as an oviposition substrate and was replaced periodically.

Chemicals. The products tested were Tracer (48% spinosad, suspension concentrate, Dow AgroSciences LLC) and RH-2485 (24% methoxyfenozide, suspension concentrate, Rohm & Haas Company, Barcelona, Spain).

Ovicidal Activity and Larval Mortality. Samples of 120–180 eggs of two different age classes, young (<24 h old) and old (24–48 h old), were collected from <7-d-old female *S. littoralis.* Filter papers strips containing egg masses were cut into small circles (≈15 mm in diameter) and dipped for 3 s in one of seven different concentrations of each insecticide (0.1–500 mg [AI]/liter). These concentrations were diluted in water, and an identical set of concentrations was diluted using analytical grade acetone. Adán et al. (1996) pointed out that organic solvents facilitate deposition and penetration of the insecticide into the insect cuticle. For this reason, spinosad and methoxyfenozide were diluted in acetone.

After drying at room temperature, the circles of filter paper containing treated egg masses were transferred individually to ventilated plastic petri dishes. Each treatment was replicated four times, and each replicate consisted of a single filter paper circle. Solvent and untreated controls were included in the assays. The number of larvae that hatched from each treatment was recorded daily after the fourth day. If sufficient larvae were available, 40 from each treatment were selected randomly from larvae that emerged on the first day of hatching. Newly hatched larvae were placed on semisynthetic diet, kept in four ventilated plastic boxes, and were observed daily to determinate larval mortality.

Larval Toxicity by Ingestion. Newly molted (0–8-h) third instars of *S. littoralis* were continuously fed semisynthetic diet containing different concentrations of spinosad and methoxyfenozide. Serial dilutions involving nine different concentrations from 0.01 to 100 mg (AI)/kg (wet weight) diet of both insecticides were prepared in 20 ml of water and mixed with the diet during its preparation (Budia et al. 1994). Untreated semisynthetic diet was provided to controls. Larvae were placed in ventilated plastic boxes (9 cm in diameter, 3 cm in height) containing two cubes (≈3 cm^3) of treated or control diet. Ten larvae per concentration were used. Larval mortality was scored daily; if no movement was observed, larvae were considered to be dead. The assay was performed four times.

Topical Treatment of Pupae. Young pupae (<24 h old) were dosed topically on the notum dorsum with 1 μl of insecticide dissolved in acetone, by using a manual microapplicator (Burkard, Hertfordshire, England). Five different concentrations of each insecticide ranging from 0.1 to 1000 mg (AI)/liter were used to treat 10 pupae per concentration (equivalent to doses of 4.5×10^{-2} to 4.5 μg [AI]/g pupa). Four replicates were used. Control specimens were treated with 1 μl of acetone alone. Pupal weight averaged (mean ± SE) 224 ± 26 mg. After treatment, we recorded mortality of pupae, adult emergence, and the prevalence of deformed adults. Pupae were considered dead if adults did not emerged after 12 d.

Data Analysis. Ovicidal activity, mortality of newly hatched larvae from treated egg masses, and effects on pupa were subjected to logit analysis and the Fieller macro present in the Generalized Linear Interactive Modeling (GLIM) program with a binomial distribution (Numerical Algorithms Group 1993). The residual analysis was calculated using the model checking macros present in the GLIM package. Scaling was performed to correct the overdispersion observed in eggs treated with insecticides dissolved in acetone. This overdispersion was corrected by the Hinkley et al. (1990) method by using the χ^2 value as scale parameter divided by the degrees of freedom. In the ingestion assay, mortality data were subjected to probit regression using the POLO-PC program (LeOra Software 1987). The lethal concentration values were calculated in milligrams of active ingredient per kilogram of diet. A range of between four and eight concentrations were used for the determination of LC_{50} values. Failure of 95% to overlap was used as criterion for significant difference at LC_{50}.

Table 1. Ovicidal activity of spinosad and methoxyfenozide, diluted in acetone, on prevalence of eclosion of *S. littoralis* eggs of two different ages

Insecticide	<24-h-old eggs					24–48-h-old eggs				
	Slope ± SE	A	LC_{50}[a]	χ^2	df	Slope ± SE	A	LC_{50}[a]	χ^2	df
Spinosad	0.36 ± 0.03	−0.45	3.43(1.69–6.45)	131.07	1	0.28 ± 0.04	−0.08	1.35 (0.31–4.22)	43.24	1
Methoxyfenozide	0.26 ± 0.07	−0.87	27.02(5.30–154.50)	15.05	1	0.13 ± 0.06	−0.74	304.29(20.57–9.66E167)	4.08	1

Parameters obtained from GLIM program (Numerical Algorithms Group 1993). Seven concentrations plus control were used for each insecticide.

[a] Concentration (95% CL) in milligrams of active ingredient per liter.

Results

Ovicidal Effects and Larval Mortality. Spinosad and methoxyfenozide, diluted in water, did not exhibit any ovicidal activity against to *S. littoralis*, after dipping young (<24-h-old) and old (24–48-h-old) eggs. For both insecticides and both egg ages, hatching percentages were between 80 and 89% and were not significantly different from those of the controls (86–87%) [($\chi^2 = 0.01$, df = 1, $P = 0.92$ and $\chi^2 = 2.70$, df = 1, $P = 0.10$ for spinosad and methoxyfenozide, respectively, for <24-h-old eggs) ($\chi^2 = 1.33$, df = 1, $P = 0.25$ and $\chi^2 = 2.51$, df = 1, $P = 0.11$ for spinosad and methoxyfenozide, respectively, for 24–48-h-old eggs)].

In contrast, when insecticides were diluted in acetone, ovicidal activity was observed in both egg age classes, although this activity was different in each compound (Table 1). For <24-h-old eggs, no significant differences were observed between LC_{50} values of spinosad (3.43 mg [AI]/liter) and methoxyfenozide (27.02 mg [AI]/liter) at 4 d after treatment, whereas LC_{50} value of spinosad (1.35 mg [AI]/liter) was 225.4-fold lower than methoxyfenozide (304.29 mg [AI]/liter) for 24–48-h-old eggs.

Mortality of larvae that hatched from both egg age classes was significantly increased in all concentrations of both insecticides compared with the controls when diluted in water or acetone alike (Table 2). Total larval mortality was scored at the second and third day after eclosion for spinosad and methoxyfenozide, respectively. When spinosad and metoxhyfenozide were diluted in water, LC_{50} values obtained for larvae that hatched from <24-h-old eggs (0.29 and 0.64 mg [AI]/liter spinosad and metoxhyfenozide, respectively) and 24–48-h-old eggs (0.26 and 0.57 mg [AI]/liter spinosad and metoxhyfenozide, respectively) were not statistically different. Likewise, when insecticides were diluted in acetone, no significant differences were observed between LC_{50} values of larvae that hatched from <24-h-old eggs (0.20 and 0.48 mg [AI]/liter spinosad and metoxhyfenozide, respectively). In contrast, LC_{50} value of spinosad (0.15 mg [AI]/liter) was 3.73-fold lower than methoxyfenozide (0.56 mg [AI]/liter) for larvae that hatched from 24- to 48-h-old eggs. Mortality of control larvae did not exceed 5% in any treatment.

Larval Toxicity by Ingestion. Third instars of *S. littoralis* were susceptible to insecticides incorporated into the diet. The LC_{50} value of spinosad (2.11 mg [AI]/kg diet) was approximately one-half that of methoxyfenozide (3.98 mg [AI]/kg diet) at 48 h after treatment (Table 3), although these values were not significantly different.

Effects on Pupal and Adult Development. When applied topically to pupae of *S. littoralis*, spinosad showed no harmful effects on pupae (7.5–12.5% mortality; $\chi^2 = 1.45$, df = 1, $P = 0.228$) or the prevalence of deformed adults (2.5–7.5%; $\chi^2 = 2.28$, df = 1, $P = 0.130$). In contrast, high concentrations of methoxyfenozide (100 and 1000 mg [AI]/liter) caused significant pupal mortality (20.0–32.5%; $\chi^2 = 9.19$, df = 1, $P = 0.002$) and deformed adults (55–65%; $\chi^2 = 61.47$, df = 1, $P = 4.50E\text{-}15$). Pupal mortality and deformed adults control resulted in 6.2 and 2.5%, respectively, for each insecticide.

Table 2. Effect of spinosad and methoxyfenozide on mortality of larvae hatching from eggs of two different age classes: young (<24 h old) and old (24–48 h old) treated with insecticides diluted in water and acetone

Insecticide	<24-h-old eggs					24–48-h-old eggs				
	Slope ± SE	A	LC_{50}[a]	χ^2	df	Slope ± SE	A	LC_{50}[a]	χ^2	df
Diluted in water										
Spinosad	0.99 ± 0.14	1.19	0.29(0.18–0.49)	110.70	1	1.04 ± 0.15	1.38	0.26(0.16–0.43)	115.7	1
Methoxyfenozide	0.87 ± 0.12	0.38	0.64(0.38–1.11)	95.75	1	0.77 ± 0.09	0.43	0.57(0.32–0.98)	130.4	1
Diluted in acetone										
Spinosad	1.20 ± 0.18	1.88	0.20(0.13–0.32)	127.7	1	1.00 ± 0.14	1.86	0.15(0.09–0.25)	108.6	1
Methoxyfenozide	0.82 ± 0.10	0.59	0.48(0.28–0.82)	138.5	1	0.74 ± 0.04	0.41	0.56(0.32–0.99)	125.9	1

Parameters obtained from GLIM program (Numerical Algorithms Group 1993). Except for larvae that hatched from <24-h-old eggs and when methoxyfenozide was diluted in acetone, three concentrations were used, and for the rest, three and four concentrations plus control were used for spinosad and methoxyfenozide, respectively.

[a] Concentration (95% LC) in milligrams of active ingredient per liter.

Table 3. Probit regression of 48-h mortality of third instars of *S. littoralis* after consumption of diet mixed with spinosad and methoxyfenozide

Insecticide	n^a	Slope ± SE	A	LC_{50} (mg [AI]/kg)	95% CL	χ^{2b}	df
Spinosad	8	3.73 ± 0.36	−1.21	2.11	1.87–2.35	1.51	6
Methoxyfenozide	4	0.86 ± 0.11	−0.51	3.98	0.82–3.64	4.18	2

Parameters obtained from POLO-PC program (LeOra Software 1987).
[a] Number of insecticide concentrations used.
[b] Goodness-of-fit χ^2 test.

Discussion

Little information is available about the ovicidal effects of spinosad and methoxyfenozide. Both insecticides showed ovicidal activity on *S. littoralis* only when they were dissolved in acetone. We assume that this solvent facilitated the penetration of each insecticide through the egg chorion to produce the observed effects. In contrast, when these insecticides were dissolved in water, neither showed any ovicidal effect. Ovicidal activity of spinosad in lepidopteran pests also has been observed in *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) (Bret et al. 1997, Peterson et al. 1998). These latter studies compared the magnitude of the ovicidal effect caused by spinosad with that of several conventional insecticides used to control these insects.

Adán et al. (1996) observed that spinosad did not have an ovicidal effect on eggs of the fruit fly *Ceratitis capitata* (Wiedemann) when they were treated with concentrations ranging from 1 to 1000 mg (AI)/liter. Similarly, Medina et al. (2001), reported a lack of toxicity of this insecticide to eggs of the predator *Chrysoperla carnea* (Stephens).

Methoxyfenozide exhibits ovicidal activity on recently laid eggs of some lepidopteran species when deposited on the treated parts of the plants (Rohm & Haas Company 1999). We observed a clear effect of this compound when dissolved in acetone. Dipping eggs of *Ostrinia nubilalis* (Hübner) and *Diatraea grandiosella* Dyar in tebufenozide and methoxyfenozide dissolved in a mixture of water + acetone (1:1) resulted in ≈100% mortality at concentrations of 100 and 200 mg (AI)/liter in both species (Trisyono and Chippendale 1997, 1998). In another study, tebufenozide dissolved in a mixture of water and acetone resulted in mortality of eggs of *Diatraea saccharalis* (F.) (Rodríguez et al. 2001), similar to that observed in our study. Acetone seems to penetrate the eggshell. In contrast, Gobbi (1998), observed that very high concentrations (10,000 mg [AI]/liter) of tebufenozide reduced the eclosion of 1-d-old eggs of *S. littoralis* to just 5%, although no toxic effect was observed in older eggs.

No evidence of ovicidal activity of tebufenozide was reported on *Lobesia botrana* Dennis & Schiffermüller and *Eupoecilia ambiguella* Hübner, regardless the age of the treated eggs (Charmillot et al. 1994). Pons et al. (1999) observed a significant increase of ovicidal activity of this insecticide in *Cydia pomonella* (L.), when oviposition occurred on apple leaves instead of filter paper.

Both insecticides dramatically reduced the survival of larvae hatched from treated eggs. Concentrations ≥10 mg (AI)/liter of spinosad or methoxyfenozide caused ≈100% mortality 2 or 3 days after hatching, irrespective of egg age or type of solvent. This probably reflects that hatching larvae can be contaminated by these compounds as they chew their way out of the egg via the mouthparts or by contact with the treated surface. Peterson et al. (1998) and Bret et al. (1997) reported similar effects in larvae of *H. virescens* and *H. zea* that emerged from spinosad-treated eggs. In contrast, Gobbi (1998) observed 90–100% mortality of *S. littoralis* at 2 d posthatching after treatment with high concentrations (100 and 10,000 mg [AI]/liter) of tebufenozide dissolved in water or acetone.

The high toxicity of spinosad via ingestion has been reported in several studies. The LC_{50} value for second instars of *Spodoptera frugiperda* (J.E. Smith) fed on surface-contaminated semisynthetic diet was 3 mg (AI)/kg (Méndez et al. 2002) very similar to the value we estimated for *S. littoralis* (2.1 mg [AI]/kg). Payne et al. (1999) reported that spinosad caused a higher prevalence of mortality than several synthetic insecticides after ingestion by first and second instars of *H. virescens* originating from >10 field strains of this species.

In surface-contaminated diet bioassays, Mascarenhas et al. (1998) reported LC_{50} values of <5 mg (AI)/liter in 2-d-old larvae of field strains of *Spodoptera exigua* (Hübner), whereas Mascarenhas and Boethel (1997) estimated LC_{50} values of ≈15 mg (AI)/liter in third instars of *Pseudoplusia includens* (Walker).

The insecticidal properties of MACs have been reported in several studies (Dhadialla et al. 1998, Smagghe and Degheele 1998). Methoxyfenozide is member of this group with particularly high activity (Ishaaya et al. 1995, Moulton et al. 2002). Methoxyfenozide was almost 7 and 4 times more toxic than tebufenozide in first instars of *S. littoralis* and neonate *D. grandiosella*, respectively (Ishaaya et al. 1995, Trisyono and Chippendale 1998). Methoxyfenozide was also far more toxic than tebufenozide in third instars of *S. exigua* (Moulton et al. 2002). Differences in the toxicity of these ecdysone agonists seem to reside in their binding affinity to ecdysone receptors (Smagghe et al. 1996).

Young pupae of *S. littoralis* were not affected by topical treatment with spinosad, possibly due to low penetration by this compound. Medina et al. (2001) also reported that spinosad was harmless to pupae of

C. carnea, although they attributed the lack of effect to the presence of the silken pupal cocoon, which probably represents an effective barrier to insecticide penetration.

Topical treatment of pupae with methoxyfenozide may interfere in the development of the imaginal discs, which normally give rise to the different adult appendages. Carton et al. (1998) injected this insecticide into pupae of *S. exigua* and observed pupal death and adult deformities. Similarly, topical treatment of *S. littoralis* pupae (>48 h old) with tebufenozide resulted in 50–65% mortality and a high prevalence of deformity of adults that emerged from treated pupae (Gobbi 1998).

Normally, the ecdysteroids induce differentiation of the adult appendages (Oberlander 1985, Riddiford 1985). We observed obvious malformations of the wings in adults that emerged from pupae treated with methoxyfenozide, but no abnormalities were noted in the legs, antennae, or any other adult body parts. Chandler et al. (1992) and Trisyono and Chippendale (1998) observed malformations of the wings in adults derived from larvae of *H. zea* and *D. grandiosella* treated with tebufenozide and methoxyfenozide, respectively. It seems that wing development is particularly sensitive to MAC compounds.

Studies performed with tebufenozide in beneficial organisms such as the immature stages of *C. carnea* and *Hyposoter didymator* (Thunberg) showed no harmful effects (Medina et al. 2001; Schneider et al. 2000, 2003a, b). The observed specificity could be due to the mode of action of this insecticide, which involves binding to the ecdysteroid receptors of Lepidoptera, whereas the receptors of other insects are less affected (Silhacek et al. 1990, Dhadialla et al. 1998, Schneider et al. 2003a)

The results obtained in this study indicate that spinosad and methoxyfenozide are potentially potent compounds for controlling *S. littoralis.* The high activity of both compounds to lepidopteran pests, along with their low toxicity to mammals (Dow Agrosciences 2002, Palli and Retnakaran 2001), may mean that these insecticides potentially represent important components in IPM programs in cotton, vegetables, and ornamentals. However, spinosad, should be used carefully, because some natural enemies, especially hymenopteran parasitoids, seem to be susceptible to this insecticide (Schneider et al. 2003a, b; Williams et al. 2003). At present, *S. littoralis* is effectively controlled with benzoylphenyl ureas (chitin synthesis inhibitors), but this pest has developed resistance to the majority of conventional insecticides. Therefore, the use of insecticide chemistries with different modes of action, such as those evaluated in the current study, should form part of an insecticide resistance management program to avoid the development of this phenomenon.

Acknowledgments

S.P. received a predoctoral fellowship from Agencia Española de Cooperación Internacional-Spain and Consejo Nacional de Ciencia y Tecnologia-Mexico. M.I.S. received a predoctoral grant from Consejo Nacional de Investigaciones Científicas y Técnicas-Argentina. We thank Trevor Williams (El Colegio de la Frontera Sur, Mexico, and Universidad Pública de Navarra, Spain) for constructive criticism. We acknowledge the comments of an anonymous reviewer, which have contributed to improve this manuscript.

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Received 12 November 2003; accepted 4 August 2004.